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PROHEXADIONE-CALCIUM (BX-112)

Ames assay (§84-2)

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DATA EVALUATION RECORD

STUDY TYPE: Bacterial reverse mutation test
OPPTS Number: 870.5100 OPP Guideline No.: §84-2

DP BARCODE: D246707 SUBMISSION CODE: S543930
P.C. CODE: 112600 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): BX-112 Technical (89.8% a.i.)

SYNONYMS: Calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid

CITATION: Jones, E. and L.A. Wilson. (1997) Bacterial Reverse Gene Mutation Assay of BX-112 Technical. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, PE 18 6ES, England. HRC Project No. KCI 25/881559, BASF Registration Document No. 91/10807. MRID 44457765. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In two trials of a reverse gene mutation assay in bacteria (MRID 44457765), *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, and *E. coli* strain WP2 uvrA were exposed to prohexadione-calcium (BX-112 Technical, 89.8% a.i.) in water, at concentrations of 312.5, 625, 1,250, 2,500, and 5,000 μg/plate in the presence and absence of mammalian metabolic activation. Appropriate solvent and positive controls were included.

Prohexadione-calcium was tested up to the limit concentration, 5,000 μg/plate, and was not toxic to strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, or WP2 uvrA in the absence or presence of metabolic activation. **Prohexadione-calcium was not mutagenic to *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, or WP2 uvrA with or without metabolic activation at up to 5,000 μg/plate.** The positive controls did induce the appropriate responses in the corresponding strains.

This study is classified as **acceptable (§84-2).** It does satisfy the requirement for FIFRA Test Guideline for in vitro mutagenicity (bacterial reverse gene mutation) data.

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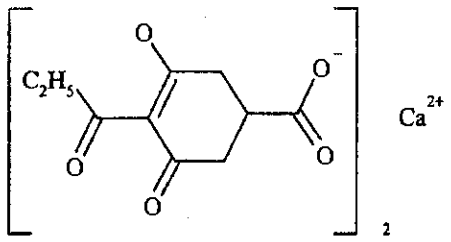
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: BX-112 Technical
   Description: Pale yellow powder
   Lot/Batch #: G14-04
   Purity: 89.8% a.i.
   Stability of compound: Expiration date reported to be September 1989; stored at 4°C in the dark. The study was conducted between September 20, 1988 and October 10, 1988.
   CAS #: 127277-53-6
   Structure:



   Solvent used: Water
   Other comments: The test substance did not dissolve in any of the solvents tested but formed a stable suspension in water.

2. Control Materials:
   Negative: Solvent served as negative control
   Solvent/final concentration: Water/0.1 mL/plate
   Positive: Nonactivation:
   Sodium azide NA[a] μg/plate TA100, TA1535
   2-Nitrofluorene 1 μg/plate TA98
   2-Nitrofluorene 2 μg/plate TA1538
   9-Aminoacridine 80 μg/plate TA1537
   Other: NA
   N-ethyl-N'-nitro-N-nitrosoguanidine 3 μg/plate TA100
   N-ethyl-N'-nitro-N-nitrosoguanidine 2 μg/plate WP2 uvrA
   N-ethyl-N'-nitro-N-nitrosoguanidine 5 μg/plate TA 1535

   [a] NA = Not Applicable

   Activation:
   2-Aminoanthracene (2-anthramine) 0.5 μg/plate *S. typhimurium* TA98 and TA1538
   2-Aminoanthracene 1 μg/plate *S. typhimurium* TA100
   2-Aminoanthracene 2 μg/plate *S. typhimurium* TA1535 and TA1537

2-Aminoanthracene __20__ μg/plate *E. coli* WP2 uvrA
Other (list): NA

3. Activation: S9 derived from

_x_ Aroclor 1254	_x_ induced	_x_ rat	_x_ liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none	_ hamster	_ other	
_ other	_ other		

The S9 mix was derived from rats pretreated with Aroclor 1254 and 1 mL of S9 mix contained the following: 0.1 mL S9 fraction (10% v:v), 8 μmol $MgCl_2$, 33 μmol KCl, 4 μmol NADH, 4 μmol NADPH, 5 μmol glucose-6-phosphate, and 100 μmol phosphate buffer (pH 7.4). The final concentration of S9 in culture was approximately 2%.

4. Test organisms: S. typhimurium strains
_ TA97 _x_ TA98 _x_ TA100 _ TA102 _ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538 ; list any others: *E. coli* WP2 uvrA
Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used *(preliminary cytotoxicity test, if performed and main assay)*:

a. Preliminary cytotoxicity test

Nonactivated conditions: 5, 50, 500, and 5,000 μg/plate TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2 uvrA, one plate/dose

Activated conditions: 5, 50, 500, and 5,000 μg/plate TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2 uvrA, one plate/dose

b. Gene mutation assay

Nonactivated conditions: 312.5, 625, 1,250, 2,500, and 5,000 μg/plate TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2 uvrA, three plates/dose

Activated conditions: 312.5, 625, 1,250, 2,500, and 5,000 μg/plate TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2 uvrA, three plates/dose

B. TEST PERFORMANCE

1. Type of Salmonella assay:
_x_ standard plate test

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_ pre-incubation (_ minutes)
_ "Privat" modification *(i.e. azo-reduction method)*
_ spot test
_ other *(describe)*

2. Protocol

a. Preliminary cytotoxicity test

A 0.1 mL aliquot of a bacterial culture containing approximately 2 x 10^9 cells/mL and 0.5 mL S-9 mix or 0.5 mL 0.1 M sodium orthophosphate buffer were placed in a glass bijou bottle. A 0.1 mL aliquot of the test solution was added to the bottle, followed by 2 mL of histidine or tryptophan deficient agar. The mixture was thoroughly shaken and overlaid onto previously prepared plates containing 25 mL of minimal agar. Single plates were used for each dose level and solvent controls were included. The plates were incubated at 37°C for 72 hours, then examined for the appearance of a complete bacterial lawn and scored for revertant colonies. Toxicity was assessed as a substantial reduction in revertant colony counts or by the absence of a complete background bacterial lawn.

b. Gene mutation assay

A 0.1 mL aliquot of bacterial suspension and 0.5 mL of sterile 0.1 M sodium orthophosphate buffer (pH 7.4) were added to each of one set of sterile bijou bottles (3 bottles/dose). A 0.1 mL aliquot of the test substance was added to each bottle at one of the five test concentrations separated by a two-fold interval. A 2 mL aliquot of histidine or tryptophan deficient agar was added to each bottle, thoroughly mixed, and overlaid onto previously prepared plates containing 25 mL of minimal agar. Three plates were used for each test concentration. Solvent and positive controls were included. The plates were incubated at 37°C for 72 hours. For metabolic activation cultures, 0.5 mL of S9 mix was substituted for the phosphate buffer. Solvent and positive controls were included. The plates were scored for revertant colonies. A positive test resulted if there was a reproducible, statistically significant (not defined), dose-related increase in the number of revertant colonies.

II. REPORTED RESULTS

A. Analytical Determinations

Analytical determinations of the dose formulations were not performed.

B. Preliminary cytotoxicity assay

One trial was conducted with prohexadione-calcium at four concentrations ranging from 5 to 5,000 μg/plate with and without metabolic activation. The tester strains used were TA 98, TA 100, TA 1535, TA 1537, TA 1538, and WP2 uvrA. Single plates were used per dose. The test compound was not toxic to any of the tester strains. The results of the preliminary cytotoxicity assay are presented in study report Table 1, page 9.

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C. Mutagenicity assay

Two trials were conducted with prohexadione-calcium at five concentrations ranging from 312.5 to 5,000 μg/plate with or without metabolic activation (Aroclor1254-induced rat livers). The tester strains used were TA 98, TA 100, TA 1535, TA 1537, TA 1538, and WP2 uvrA. Three plates were used per dose. No mutagenic activity was observed in the solvent control. No substantial increase in mutagenic activity was observed in revertant colonies of any of the six tester strains treated with the test compound at any dose level either with or without metabolic activation. The positive controls gave the appropriate responses. The individual plate results of the gene mutation assay are presented in study report Tables 3, 4, 6, and 7, pages 11, 12, 13, 15, 16, and 17). The results are summarized in study report Tables 2 and 5, pages 10 and 14, and are presented as an Appendix to this DER.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. Prohexadione-calcium was assayed in six *S. typhimurium* strains with and without S9 at concentrations up to the limit dose. The test compound was not cytotoxic, and failed to induce a genotoxic response. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls.

B. Study deficiencies - Data on the analysis of dose formulations for actual concentration were not submitted. However, as the test compound was tested up to the limit dose, this deficiency is not expected to affect the interpretation of this study. Several minor deficiencies noted in the study that are not considered to affect the validity of the study results are:

- Criteria for statistical significance were not provided.
- Historical control ranges (solvent and positive) were not provided.

DER Bacterial Reverse Mutation
MRID 44457765

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